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RRL 3/9/88 006652

DATA EVALUATION REPORT

Study Type: Combined Chronic Toxicity/Oncogenicity (Mouse)

TOX Chem No.: 634
Project No.: 7-0855

Accession No.: 402024-03

Test Material: Paraquat (1,1'-Dimethyl-4,4'-bipyridinium dichloride)

Synonyms: AT-5, Parakote, Paracote

Study Number(s): None

Sponsor: Asahi Chemical Industry Company, Ltd.
Japan

Testing Facility: Nippon Experimental Medical Research Institute
Japan

Title of Report: AT-5: Chronic Toxicity Study Result - 104-Week Dosing Study in Mouse

Author(s): S. Toyoshima, R. Sato, M. Kashima, and M. Motoyama

Report Issued: March 10, 1982

Conclusions:

Systemic NOEL = 30 ppm (3.92 mg/kg/day*, males)
Systemic NOEL = 30 ppm (3.82 mg/kg/day*, females)
Systemic LEL = 100 ppm (13.09 mg/kg/day*, males and females; HDT); (increased mortality rate in females; decreased total protein, RBC, hemoglobin, hematocrit, and leukocytes in males and females; decreased polymorphonucleocytes in males and GPT and alkaline phosphatase activities in females; increased blood glucose in males and females; decreased absolute and/or relative weights of adrenals, thyroid, liver, and urinary bladder in males; decreased absolute weight of brain in females; and increased absolute and/or relative weights of kidneys, lungs, and heart in males).

Oncogenic NOEL = > 100 ppm (males and females; HDT)

*Values reported by the testing laboratory.

Gross, non-neoplastic, and neoplastic lesions were observed in various organs of males and females, but did not appear to be treatment related. The most frequent lesions were observed in the lungs (hepatoid changes, congestion; nodes, pneumonia, thickening of alveolar walls, and adenocarcinoma, all in both sexes); liver (turbidity in both sexes, dilatation in females and tumors in males); kidneys (discoloration and coarse surface in both sexes; renal pelvis dilatation and cell infiltration in males; and nephropathy in females); spleen (swelling in both sexes and turbidity and dilatation mostly in females); thymus (atrophy in both sexes and hypertrophy in females); mesenteric lymph node (swelling and cell infiltration in both sexes); eyes (corneal cell proliferation in both sexes and corneal calcification in females); testes (calcification of seminiferous tubules); seminal vesicles (hypertrophy); mammary gland (atrophy and cysts); ovaries (hematoma, cysts, edema, atrophy, and hypoplasia of corpora lutea and follicles); and uterus (edema, atrophy, cysts, and polyps). Leukemia, amyloid degeneration, and leukemia cell infiltration were also observed frequently in males and females.

Classification: Chronic feeding study: Core-Supplementary*
Oncogenic Study: Core-Supplementary*

*Because of omissions and ambiguities, detailed in the review.

A. Materials:

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1. Test compound - Technical-grade paraquat dichloride supplied by the sponsor; purity: at least 98%; colorless crystalline powder, readily soluble in water; lot no. 540108.

2. Test animals - Three-week-old JCL:ICR mice, purchased from Japan Clea Laboratories Company, Ltd., Tokyo. Acclimation period: 1 week. Body weight at the initiation of study: 25 to 29 g (males) and 23 to 26 g (females).

B. Study Design:

1. Animal assignment - Animals were assigned randomly to the following test groups:

Test Group	Dose in Diet (ppm)	Main Study 104 Weeks		Interim Sacrifice 26 Weeks		52 Weeks	
		Males	Females	Males	Females	Males	Females
I	0	60	60	10	10	10	10
II	2	60	60	10	10	10	10
III	10	60	60	10	10	10	10
IV	30	60	60	10	10	10	10
V	100	60	60	10	10	10	10

Dose levels used, expressed as paraquat dichloride, were based on the results of preliminary studies (not submitted). Animals were housed 5/sex/cage at 22 °C and relative humidity of 55 percent. The shelves equipped with 30 cages each were rotated to the left of the animal room once every 2 months. Each cage was likewise moved one level down and the lowest level was transferred to the highest level to maintain uniform experimental conditions.

2. Diet preparation - Diet was prepared by Japan Clea Laboratories once every 5 months and pelleted. For every 100 kg of food, 100 g was sent to the sponsor for analysis of paraquat content. At the testing facility, diets were stored at 5 °C and were tested for paraquat stability.

Results - The analytical concentrations of paraquat dichloride in diets ranged from 102 to 106.5 percent of the theoretical (nominal) concentrations.

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Paraquat was stable in diets after storage for 5 months at 5 °C. The concentrations of paraquat dichloride in diets ranged from 95 to 103 percent of the initial concentrations.

The stability of paraquat in diets at room temperature was not tested.

3. Animals received food (Solid Food CE-2 manufactured by Japan Clea Laboratories Company, Ltd.) and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data:

   Student's t-test: Body weight, hematology, serum biochemistry, and organ weights. Chi-square test: Mortality and incidence of tumors.

   The following levels of significance were used: *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$).

5. Quality assurance statement was not submitted. This study was originally reported in Japanese. ICI Americas, Inc., who submitted the English translation as well as the original report, also included the following Good Laboratory Practice statement:

   > The submitter of this study was neither the sponsor of this study nor conducted it, and does not know whether it has been conducted in accordance with 40 CFR Part 160.

C. Methods and Results:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality. After test week 26, each animal was palpated weekly for masses.

   Results - Lowered spontaneous mobility, loss of coat luster, and piloerection were observed in moribund animals.

   There were no deaths during the first year of study. At the termination of the study, the mortality rate in the 100 ppm female group was 13 percent higher than that in

the control group. The mortality rates in other treated groups were similar to those in the control groups. These data are summarized below.

Incidence of Mortality

Paraquat Dichloride (ppm)	Males					Females				
	0	2	10	30	100	0	2	10	30	100
Weeks	Number of Animals									
1 - 26	0	0	0	0	0	0	0	0	0	0
27 - 52	0	0	0	0	0	0	0	0	0	0
53 - 60	0	1	0	1	1	0	1	1	1	0
61 - 70	4	8	8	5	5	2	4	2	5	4
71 - 80	11	16	20	15	17	13	10	10	13	13
81 - 90	26	28	34	27	28	23	23	17	27	30
91 - 100	42	35	40	39	37	29	33	28	37	38
101 - 104	43	37	42	43	42	34	38	32	39	42
Survivors	17	23	18	17	18	26	22	28	21	18
Weeks	Percent Incidence*									
1 - 26	0	0	0	0	0	0	0	0	0	0
27 - 52	0	0	0	0	0	0	0	0	0	0
53 - 60	0	1.7	0	1.7	1.7	0	1.7	1.7	1.7	0
61 - 70	6.8	13.3	13.3	8.3	8.3	3.3	6.7	3.3	8.3	6.7
71 - 80	18.3	26.7	33.3	25	28.3	21.7	16.7	16.7	27.7	21.7
81 - 90	43.3	46.7	56.7	45	46.7	38.3	38.3	28.3	45	50
91 - 100	70	58.3	66.7	65	61.7	48.3	55	46.7	61.7	63.3
101 - 104	71.7	61.7	70	71.7	70	56.7	63.3	53.3	65	70
Survivors	28.3	38.3	30	28.3	30	43.3	36.7	46.7	35	30

*The interim sacrifices were excluded from calculating incidence.

Amyloid degeneration in various organs, pneumonia, pulmonary adenocarcinoma, leukemia, and leukemia cell infiltration into various organs were reported as major causes of deaths in both sexes and at all dose levels, including controls. In the 2 and 10 ppm female groups, nephropathy was also reported as a major cause of death.

2. Body weight - Animals were weighed weekly from the initiation of study until week 26 and every 2 weeks thereafter.

Results - Body weight gains were not inhibited in any group when the treated animals were compared with the controls. Slight but statistically significant ($p < 0.01$, 0.01, or 0.001) increases in mean body weights in the 30 ppm females (2 to 7% over those of controls) did not appear to be treatment-related. These weight

increases occurred only during the first year (weeks 3 to 4, 7 to 15, 17, 23 to 25, 30, and 42 to 52) and were not observed in the 30 ppm males or 100 ppm males and females.

3. Food consumption and compound intake - Consumption was determined weekly from the initiation of study until week 26 and every 2 weeks thereafter, and mean daily consumption per animal was calculated. Food efficiency (ratio of mean body weight gain to mean food consumption) for each sex was calculated at weeks 26, 52, and 104. Compound intake was calculated weekly from the food consumption and body weight data until week 26 and every 2 weeks thereafter.

   Results - Paraquat had no effect on food consumption and food efficiency when the treated animals were compared with the controls. The compound intake, calculated as paraquat dichloride, was as follows:

Concentration in Diet (ppm)	Mean Intake (mg/kg of Body Weight/Day)	
	Males	Females
2	0.26	0.26
10	1.31	1.32
30	3.92	3.82
100	13.09	13.03

   According to these data, there was no difference between males and females in the ingestion of the test material at each dose level.

4. Ophthalmological examinations were not performed.

5. Blood was collected from 10 males and 10 females in each group before sacrifice at test weeks 26 and 52, and from all survivors at test week 104. The checked (X) parameters were examined.

   a. Hematology

X	Hematocrit (HCT)*		Total plasma protein (TP)
X	Hemoglobin (HGP)*	X	Leukocyte differential count
X	Leukocyte count (WBC)*		Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)*		Mean corpuscular HGB conc. (MCHC)
X	Platelet count*		Mean corpuscular volume (MCV)

*Recommended by Subdivision F (October 1982) guidelines for chronic studies.

Results - Statistically significant ($p < 0.05$) decreases in various parameters were observed only in the 100 ppm male and female groups as follows:

Males

- Erythrocytes (6.1 to 7.7%) and hematocrit (4.1 to 7.4%) at all time intervals tested.
- Leukocytes at weeks 26 and 52 (20.5 and 31.4%, respectively).
- Hemoglobin (4.3%) and lymphocytes (9.2%) at week 26.
- Polymorphonucleocytes (13.5%), at week 104.

Females

- Hemoglobin (6.8 to 10.0%) at all time intervals tested.
- Leukocytes (17.8%) at week 26.
- Erythrocytes (7.8%) at week 52.
- Hematocrit (8.7%) at week 104.

b. Clinical Chemistry

X	Electrolytes:
	Calcium*
X	Chloride*
	Magnesium*
	Phosphorous*
X	Potassium*
X	Sodium*
	Enzyme Activities
X	Alkaline phosphatase
X	Cholinesterase[a]
	Creatinine phosphokinase*
	Lactic dehydrogenase
X	Serum alanine aminotransferase (also SGPT)*
X	Serum aspartate aminotransferase (also SGOT)*

X	Other:
	Albumin*
X	Blood creatinine*
X	Blood urea nitrogen*
X	Cholesterol*
	Globulins
X	Glucose*
	Total Bilirubin*
X	Total Protein*
	Triglycerides
X	Albumin/globulin ratio

[a]Brain, serum, and corpuscular cholinesterase activities were determined.

*Recommended by Subdivision F (October 1982) guidelines for chronic studies.

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Results - Statistically significant ($p < 0.05$, 0.01, or 0.001) changes in various parameters were observed only in the 100 ppm male and female groups as follows:

Males

- Decreases in total protein (7.9 to 11.8%) at all time intervals tested.
- Increase in glucose (47.4%) at week 104.

Females

- Decreases in total protein (7.4 and 9.9%) at weeks 26 and 104, respectively.
- Decreases in GPT (16.7%) and alkaline phosphatase (16.8%) activities at week 52.
- Increase in glucose (17.8 and 29.2%) at weeks 52 and 104, respectively.

6. Urinalysis - Urine was collected from 10 males and 10 females in each group before sacrifice at test weeks 26 and 52, and from all survivors at test week 104. The checked (X) parameters were examined.

	Appearance*	X	Glucose*
	Volume*	X	Ketones*
	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilinogen

Results - Paraquat had no effect on any of these parameters examined when the treated animals were compared with the controls.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X	Digestive System
	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*
	Gallbladder
XX	Pancreas*
	Respiratory
X	Trachea*
XX	Lung*

X	Cardiovasc./Hemat.
	Aorta*
XX	Heart*
X	Bone marrow*
X	Lymph nodes*
XX	Spleen*
XX	Thymus*
	Urogenital
XX	Kidneys*
XX	Urinary bladder*
XX	Testes*
	Epididymides
XX	Prostate
XX	Seminal vesicle
XX	Ovaries
XX	Uterus*

X	Neurologic
XX	Brain*
X	Periph. nerve*
	Spinal cord (3 levels)
XX	Pituitary*
X	Eyes (optic n.)*
	Glandular
XX	Adrenals*
	Lacrimal gland
	Mammary gland*
X	Parathyroids*
XX	Thyroids*
	Other
	Bone*
X	Skeletal muscle
X	Skin
X	All gross lesions and masses

Microscopic examination was done on specimens stained with hematoxylin-eosin. The lung was additionally examined for connective tissue after staining with van Gieson's stain. Eyes were examined after treatment with Bouin fixative. Organs were not weighed for animals that died during the study.

Results

a. Organ weight - Statistically significant ($p < 0.05$ or 0.01) changes in absolute and relative** organ weights were observed in the 30 and 100 ppm male groups and in the 100 ppm female group as follows:

Adrenals - Decreases in absolute (25%) and relative (24%) weights of left adrenal in 30 ppm males at week 26.

Decreases in absolute (37%) and relative (37%) weights of left adrenal in 100 ppm males at week 26.

**Relative weight = organ weight/body weight ratio.

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Thyroid - Decreases in absolute (22%) and relative (23%) weights in 100 ppm males at week 26 and in absolute weight (19%) at week 104.

Liver - Decrease in absolute weight (17%) in 100 ppm males at week 104.

Urinary bladder - Decrease in absolute weight (33%) in 100 ppm males at week 104.

Brain - Decrease in absolute weight (4%) in 100 ppm females at week 104.

Kidneys - Increased relative weight (10%) of left kidney in 100 ppm males at week 104.

Lungs - Increases in absolute (11%) and relative (9%) weights in 100 ppm males at week 26.

Heart - Increase in absolute weight (14%) in 100 ppm males at week 52.

b. Gross pathology - Very few changes were observed at the 26-week interim sacrifice, but the incidence was increased at the 52-week scheduled sacrifice.

At week 26, necropsy revealed changes in the lung, thyroid, and lymph node. These changes consisted of nodules of the lung (one male each in the 30 and 100 ppm group), nodules of the parathyroid (one male in the 10 ppm group) and swelling of the lymph nodes (one female each in the control and 2 ppm groups).

At week 52, gross changes were observed in the lung, kidney, liver, large intestine, and uterus. These changes included lung nodules, liver-like (hepatoid) changes and focal bleeding of the lung, hepatic and kidney discoloration, edema and surface coarseness of kidneys, swelling of the lymph nodes of large intestine, and uterine edema. The total numbers of males and (females) affected in the 0, 2, 10, 30, and 100 ppm groups were 2(4), 3(3), 2(4), 4(3), and 5(3), respectively.

Ten animals/sex/dose level were examined at each interim sacrifice.

The predominant gross pathological changes in animals sacrificed at the termination of the study (week 104) and in those which died or had to be sacrificed moribund during the course of the study were as follows:

Percent Incidence* of Predominant Gross Changes Observed at the Termination of the Study (Week 104)

Paraquat Dichloride (ppm)	Males					Females				
	0	2	10	30	100	0	2	10	30	100
Number of mice (organs) examined	17	23	18	17	18	26	22	28	21	18
Lungs										
Nodes	24	17	39	24	50	23	23	14	38	22
Congestion	12	17	5	29	22	8	0	4	10	6
Hepatoid changes	6	0	0	6	11	12	0	11	10	17
Tumors	0	0	0	0	0	0	0	18	5	0
Liver										
Hypertrophy	6	4	0	6	0	4	9	0	14	6
Filled with bile	0	4	0	0	0	8	9	4	10	11
Spleen										
Hypertrophy	6	0	6	12	6	19	18	7	10	6
Turbidity	0	4	0	6	0	19	14	11	14	11
Dilatation	0	0	0	18	0	19	14	11	10	6
Kidneys										
Pale appearance	29	26	5	18	5	19	18	4	0	22
Thymus										
Hypertrophy	0	0	0	0	0	27	14	11	19	6
Testis										
Soft	29	22	5	18	33	--	--	--	--	--
Seminal vesicle										
Hypertrophy	12	13	22	18	11	--	--	--	--	--

Percent Incidence* of Predominant Gross Changes Observed at the Termination of the Study (Week 104) (cont'd)

Paraquat Dichloride (ppm)	Males					Females				
	0	2	10	30	100	0	2	10	30	100
Number of mice (organs) examined	17	23	18	17	18	26	22	28	21	18
Ovary										
Cyst	--	--	--	--	--	15	5	25	10	11
Hematoma	--	--	--	--	--	4	9	7	0	17
Uterus										
Edema	--	--	--	--	--	35	27	25	24	28

*Percent incidence = Number of organs observed with changes x 100/number of organs examined.

According to the above data, gross changes were observed in various organs, but did not appear to be treatment-related. The percent incidence of these changes was either similar in the control and paraquat-treated groups or a dose relationship was lacking.

Percent Incidence* of Predominant Gross Changes Observed in Rats that Died During the Course of the Study (Unscheduled Deaths)

Paraquat Dichloride (ppm)	Males					Females				
	0	2	10	30	100	0	2	10	30	100
Number of mice (organs) examined	43	37	42	43	42	34	38	32	39	42
Lungs										
Hepatoid changes	9	8	5	0	0	3	3	13	18	5
Nodes	21	14	5	12	0	0	5	13	8	5
Congestion	14	27	31	21	31	41	39	31	13	38
Tumor	7	16	14	19	17	6	11	6	3	10

Percent Incidence* of Predominant Gross Changes Observed in Rats that Died During the Course of the Study (Unscheduled Deaths) (cont'd)

Paraquat Dichloride (ppm)	Males					Females				
	0	2	10	30	100	0	2	10	30	100
Number of mice (organs) examined	43	37	42	43	42	34	38	32	39	42
Liver										
Turbidity	16	19	12	0	5	6	13	3	5	2
Dilatation	0	0	0	0	0	6	13	3	5	2
Tumor	16	19	12	2	5	0	0	0	0	2
Spleen										
Hypertrophy	16	24	12	14	10	15	16	16	15	14
Kidneys										
Pale appearance	28	22	21	28	26	21	24	0	10	10
Rough surface	7	11	7	9	2	15	11	6	5	7
Edema	2	0	5	5	5	3	0	0	0	0
Divided surface	0	0	0	0	0	3	5	6	5	0
Skin										
Tumor	0	5	2	5	2	6	3	0	3	5
Lymph nodes										
Swelling	12	24	12	19	2	9	13	16	13	10
Uterus										
Atrophy	--	--	--	--	--	18	11	3	13	5
Edema	--	--	--	--	--	12	13	25	13	12
Ovary										
Edema	--	--	--	--	--	26	13	16	5	2

*Percent incidence = Number of organs observed with changes x 100/number of organs examined.

According to the above data, gross changes were observed in various organs, but did not appear to be treatment-related. The percent incidence of these changes was either similar in the control and paraquat-treated groups or was increased in the treated groups, but a dose relationship was lacking.

Similar pulmonary changes were observed in animals sacrificed at the termination of the study and in those that died or were sacrificed moribund during the course of the study. However, the incidence of pulmonary and skin tumors and swelling in lymph nodes (all dose-unrelated) was higher in animals that did not survive the study.

c. Microscopic pathology

1) Non-neoplastic - The predominant non-neoplastic lesions, observed at the 26-week interim sacrifice were slight to moderate thickening of alveolar walls and bronchodilatation in males and females, swelling of thymus in females, and calculi in urinary bladder in males. The incidence of these lesions ranged from 10 to 40 percent per dose level and was dose-unrelated. Thickening of alveolar walls and swelling of the thymus occurred in every group, including the controls. Bronchodilatation was observed in two males from the 10 ppm group and three males from the 100 ppm group, and in one, one, and two females from the control, 2 and 10 ppm groups, respectively. The incidence of calculi was higher in the control group than in the 30 and 100 ppm groups, where they only occurred.

The predominant non-neoplastic lesions observed in males and females at the 52-week interim sacrifice, were slight to moderate thickening of alveolar walls, cell infiltration (kidneys and salivary gland), renal pelvis dilatation, swelling and atrophy of thymus, swelling of mesenteric lymph node, calculi in urinary bladder, and uterine cysts. The incidence of these lesions ranged from 10 to 40 percent per group, but was dose-unrelated.

The incidence of predominant non-neoplastic lesions observed at the termination of the study (week 104) and in animals that died or were sacrificed moribund during the course of the study, was as follows:

Paraquat Dichloride (ppm)	Male Mice					Female Mice				
	0	2	10	30	100	0	2	10	30	100
Number of tissues examined*	Percent Incidence at Week 104**									
	17	23	18	17	18	26	22	28	21	18
Lungs										
Thickening of alveolar walls	12	30	28	47	39	31	55	61	43	28
Kidneys										
Cell infiltration	12	24	17	6	6	0	5	4	0	6
Thymus										
Atrophy	6	30	17	47	44	19	27	11	10	0
Mesenteric lymph node										
Cell infiltration	0	0	0	6	6	8	5	7	5	11
Eyes										
Corneal cell proliferation	6	24	17	6	0	4	14	4	0	6
Corneal calcification	0	0	0	0	0	4	0	7	0	6
Testes										
Calcification of seminiferous tubule	18	30	22	35	33	--	--	--	--	--
Mammary gland										
Atrophy	--	--	--	--	--	42	27	54	38	44
Cysts	--	--	--	--	--	0	14	7	5	11

Paraquat Dichloride (ppm)	Male Mice					Female Mice				
	0	2	10	30	100	0	2	10	30	100
Number of tissues examined*	Percent Incidence at Week 104**									
	17	23	18	17	18	26	22	28	21	18
Ovary										
Follicle formation insufficiency	--	--	--	--	--	4	9	11	10	11
Luteinization insufficiency	--	--	--	--	--	4	14	11	14	6
Follicle yellow body formation insufficiency	--	--	--	--	--	23	32	25	29	61
Cysts	--	--	--	--	--	31	18	18	14	11
Uterus										
Atrophy	--	--	--	--	--	0	9	11	19	6
Cysts	--	--	--	--	--	58	59	50	4	78
Number of tissues examined*	Percent Incidence in All Nonsurvivors**									
	43	37	42	43	42	34	38	32	39	42
Lungs										
Thickening of alveolar walls	35	14	12	12	19	18	24	3	10	10
Pneumonia	28	24	31	23	31	47	37	41	33	48
Spleen										
Swelling	5	3	7	7	2	3	5	0	3	0
Kidneys										
Renal pelvis dilatation	5	3	5	5	7	3	0	0	0	0
Nephropathy	0	0	0	2	0	9	18	16	8	0
Urinary bladder										
Calculi	0	0	2	5	12	0	0	0	0	1
Testes										
Calcification of seminiferous tubule	14	14	14	19	17	--	--	--	--	--

Paraquat Dichloride (ppm)	Male Mice					Female Mice				
	0	6	10	30	100	0	6	10	30	100
Number of tissues examined*	Percent Incidence in All Nonsurvivors**									
	43	37	42	43	42	34	38	32	39	42
Mammary gland										
Cysts	--	--	--	--	--	3	8	3	3	2
Ovary										
Luteinization insufficiency	--	--	--	--	--	15	5	13	13	10
Atrophy	--	--	--	--	--	3	24	34	28	24
Cysts	--	--	--	--	--	38	32	28	10	17
Uterus										
Atrophy	--	--	--	--	--	38	53	16	31	12
Cysts	--	--	--	--	--	12	21	34	18	26

*For each organ or tissue, numbers examined histologically are the same as numbers of animals examined. All animals sacrificed at week 104 and those that died during the study (unscheduled deaths) were examined.
**Percent incidence = number of tissues with lesions x 100/number of tissues examined.

According to the above data, thickening of alveolar walls, calcification of seminiferous tubules, cysts in mammary gland, ovarian cysts, and uterine atrophy and cysts were observed in the animals sacrificed at the termination of the study and in the nonsurvivors. The nonsurvivors also had pneumonia and swollen spleen (males and females), renal pelvis dilatation (mostly males), nephropathy (mostly females), and calculi in urinary bladder. The following lesions were either infrequent or absent in the nonsurvivors, but were observed in both sexes at the termination of the study: atrophy of the thymus, corneal cell proliferation, corneal calcification (females only), and atrophy of mammary gland (females only).

The above lesions did not appear to be treatment-related. In most instances, there were great variations in the incidence of lesions from one group to another and a dose-relationship was lacking.

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Compared with the controls, follicle yellow body formation insufficiency (ovarian lesion) and uterine cysts were increased in the 100 ppm females at the terminal sacrifice, whereas calculi in urinary bladder were increased with dose in the nonsurviving males. However, the incidence of the ovarian lesion did not increase with dose in the 0, 2, 10, and 30 ppm groups, and was not observed in the nonsurvivors. The incidence of uterine cysts (very common lesions) was high in the low-dose groups (2 and 10 ppm), very low in the 30 ppm group, and high again in the 100 ppm group, and did not appear to be treatment-related. Dose-unrelated calculi in urinary bladder were also observed in males at the 26-week interim sacrifice and in males and females at the 52-week interim sacrifice. The dose-related incidence of urinary bladder calculi in the nonsurviving males cannot therefore be unequivocally attributed to treatment.

Another non-neoplastic lesion observed frequently in males and females was amyloid degeneration. This lesion was first noted in the small intestine of two females (one in each 2 and 30 ppm groups) at the 26-week sacrifice. At the 52-week sacrifice, amyloid degeneration occurred mostly in the liver, spleen, kidneys, small intestine, and pancreas of the untreated males. At the terminal sacrifice and in the nonsurvivors, dose-unrelated amyloid degeneration was observed in virtually every organ or tissue examined. At the terminal sacrifice the highest incidence of amyloid degeneration occurred in the small intestine of males (33 to 67%) and females (32 to 64%). In the nonsurvivors (untreated and treated males and females) the highest incidence of amyloid degeneration occurred in the following tissues: liver (9 to 37%), spleen (12 to 33%), kidneys (16 to 48%), small intestine (21 to 56%), thyroid (8 to 40%), and adrenals (12 to 42%).

In summary, for reasons discussed above, the non-neoplastic lesions observed in this study did not appear to be treatment-related. Also, most of these lesions are common in mice, especially after 6 or 12 months of life[1].

Connective tissue, after staining lungs with van Gieson's stain, was observed only in the following animals: 1 female out of 10 examined in the 100 ppm group at the 52-week interim sacrifice; 1 male out of 23 examined (incidence = 4%) in the 2 ppm group at the terminal sacrifice; and in a few males and females that died or were sacrificed moribund during the course of the study (nonsurvivors). The percent incidence of positive findings, very slightly positive (±) or slightly positive (+), in male nonsurvivors was 2, 8, 5, 7, and 2 in the 0, 2, 10, 30, and 100 ppm groups, respectively. The corresponding values for the female groups were 0, 0, 3, 3, and 10, respectively.

Based on the above data, the incidence of this lesion (pulmonary fibrosis) was low in this study and did not appear to be treatment-related, at least in the males. Since there were no other treatment-related non-neoplastic pulmonary lesions in the 100 ppm female nonsurvivors, it could not be concluded with certainty that a slight increase in the incidence of pulmonary fibrosis was treatment-related.

2) Neoplastic - The incidence of neoplastic lesions in this study was as follows:

Paraquat Dichloride (ppm)	Male Mice					Female Mice				
	0	2	10	30	100	0	2	10	30	100
Number of tissues examined*	Percent Incidence at Week 26 Interim Sacrifice**									
	10	10	10	10	10	10	10	10	10	10
Lungs										
Adenoma				10						
Adenocarcinoma					10					

[1] Selected Nonneoplastic Diseases. J.D. Burek, J.A. Molello, and S.D. Warner. In The Mouse in Biomedical Research, Volume II; H.L. Foster, J.D. Small, and J.G. Fox, Editors; Academic Press (1982); pages 425-438.

Paraquat Dichloride (ppm)	Male Mice					Female Mice				
	0	2	10	30	100	0	2	10	30	100
Number of tissues examined*	Percent Incidence at Week 26 Interim Sacrifice**									
	10	10	10	10	10	10	10	10	10	10
Lymph node										
Lymphoma							10			
Number of tissues examined*	Percent Incidence at Week 52 Interim Sacrifice**									
	10	10	10	10	10	10	10	10	10	10
Lungs										
Adenoma			10				10			
Adenocarcinoma		10	10		20					
Uterus										
Polyp						10	10		10	
Leukemia	10									
Number of tissues examined*	Percent Incidence at Week 104 (Terminal Sacrifice)**									
	17	23	18	17	18	26	22	28	21	18
Lungs										
Fibroma		4								
Adenocarcinoma	29	30	28	23	50	31	32	43	38	44
Liver										
Angioma									5	
Adenoma		13			11	4				5
Spleen										
Angioma		4								
Fibrosarcoma										5
Testes										
Seminoma	6		11		6	--	--	--	--	--
Mesenteric lymph node										
Lymphoma				6		8				
Lymphosarcoma										5

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Paraquat Dichloride (ppm)	Male Mice					Female Mice				
	0	2	10	30	100	0	2	10	30	100
Number of tissues examined*	Percent Incidence at Week 104 (Terminal Sacrifice)**									
	17	23	18	17	18	26	22	28	21	18
Adrenals										
Tumor		4								
Bone										
Osteosarcoma										5
Skin										
Soft tumor								4		
Ovary										
Tumor	--	--	--	--	--		6			
Hypernephromoid	--	--	--	--	--			4		
Uterus										
Polyp	--	--	--	--	--	4		4	5	11
Myoma	--	--	--	--	--			7		5
Fibroma	--	--	--	--	--			4	5	
Leukemia	6			6	6	62	68	64	62	56
T-cell leukemia	6					8	5	4		
Number of tissues examined*	Percent Incidence in All Nonsurvivors**									
	43	37	42	43	42	34	38	32	39	42
Lungs										
Adenocarcinoma	28	30	21	30	17	9	16	22	10	17
Liver										
Angioma										2
Hepatoadenoma	5	5	2							
Spleen										
Angioma							3			

Paraquat Dichloride (ppm)	Male Mice					Female Mice				
	0	2	10	30	100	0	2	10	30	100
Number of tissues examined *	Percent Incidence in All Nonsurvivors**									
	43	37	42	43	42	34	38	32	39	42
Duodenum										
Carcinoma			2							
Small intestine										
Fibroadenoma										2
Muscle										
Fibroma			2							
Sarcoma										2
Bone										
Osteoma								3		2
Skin										
Fibrosarcoma				5	2	3				2
Spindle cell sarcoma		3								
Adenocarcinoma		3								
Small circular cell sarcoma										2
Squamous cell carcinoma	2									
Basal cell carcinoma							3		3	
Testes										
Seminoma	2			7	2	--	--	--	--	--
Mammary gland										
Fibroadenoma	--	--	--	--	--					2
Adenocarcinoma	--	--	--	--	--		5		3	
Ovary										
Tumor								3		
Fibroma	--	--	--	--	--					2
Carcinoma	--	--	--	--	--					2

Paraquat Dichloride (ppm)	Male Mice					Female Mice				
	0	2	10	30	100	0	2	10	30	100
Number of tissues examined	Percent Incidence in All Nonsurvivors**									
	43	37	42	43	42	34	38	32	39	42
Uterus										
Polyp	—	—	—	—	—	3		3		2
Myoma	—	—	—	—	—		3		3	2
Mesenteric lymph node										
Lymphoma								3		
Leukemia	19	30	24	28	14	50	24	44	41	33
T-cell leukemia										2

**Percent incidence = Number of tissues with lesions x 100/number of tissues examined.

Blank space = Absence of lesion.

*For each organ or tissue, numbers examined histologically are the same as numbers of animals examined.

Only three neoplastic lesions were observed at the 26-week interim sacrifice: pulmonary adenoma (30 ppm male), pulmonary adenocarcinoma (100 ppm male), and lymphoma (2 ppm female).

At the 52-week interim sacrifice, a total of 10 neoplastic lesions were noted as follows: 2 pulmonary adenomas (2 ppm female and 10 ppm male); 4 pulmonary adenocarcinomas (2 ppm, 10 ppm, and 2 in 100 ppm males); 3 uterine polyps (0 ppm, 2 ppm, and 30 ppm females); and 1 leukemia in the male control group.

At the terminal sacrifice, the most frequently observed neoplastic lesions were leukemia and T-cell leukemia in females, uterine polyps, and pulmonary adenocarcinoma in males and females. The percent incidence of leukemias and pulmonary adenocarcinoma in females was dose-unrelated, but the incidence of uterine polyps was 7 percent higher in the 100 ppm group than in the controls. The percent incidence of pulmonary adenocarcinoma in males was similar in the 0, 2, and 10 ppm groups; 6 percent lower compared with the controls in the 30 ppm group; and 21

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percent higher, compared with the controls, in the 100 ppm group. In the females, leukemia cells were found in virtually every organ or tissue examined, but were most abundant in lungs, liver, spleen, kidneys, urinary bladder, stomach, thymus, bone marrow, mesenteric lymph node, and salivary glands.

The most frequently observed neoplastic lesions in the nonsurvivors (males and females) were leukemia and pulmonary adenocarcinoma, but the percent incidence was dose-unrelated. Leukemia cells were abundant in both sexes, in the same organs as those listed above, and also in brain and eyes. However, leukemia cell infiltration was low in the thymus of females and was not observed in the thymus of males.

Paraquat dichloride did not appear to be oncogenic in this study. The only tumor of concern was an increased incidence of pulmonary adenocarcinoma in the 100 ppm males observed only at the terminal sacrifice. However, if all pulmonary adenocarcinomas observed in each group during the course of the study are related to 80 animals examined histopathologically in each group, the incidence of this lesion is as follows:

Paraquat dichloride (ppm)	Male Mice Incidence		Female Mice Incidence	
	Numerical	Percent	Numerical	Percent
0	17	21.3	11	13.8
2	19	23.8	13	16.3
10	15	18.8	19	23.8
30	17	21.3	12	15.0
100	19	23.8	12	15.0

Based on the total incidence of pulmonary adenocarcinoma, paraquat did not appear to be oncogenic in the lungs of male mice. This finding is also supported by historical control data submitted by the testing laboratory for four 2-year feeding studies (presumably with the same strain of mice). In these studies, conducted during 1976 and 1982, 99, 44, 75, or 70 mice of each sex per study were used and all were examined histologically. The incidence of

pulmonary adenocarcinoma in the males ranged from 13.3 to 27.1 percent and in the females from 11.4 to 15.2 percent. The incidence of pulmonary adenocarcinoma in the 100 ppm males was, therefore, within that observed in the historical control males. The highest incidence of tumors occurred during weeks 92 to 104.

Uterine polyps are common in mice, especially aged mice, and the slight increase in the 100 ppm groups was probably not treatment-related.

Leukemia is considered "the most common hematopoietic malignancy in the mouse"[2]. In this study, the incidence of leukemia was higher in the controls than in the 100 ppm group or in some of the other treated groups. Yet, leukemia was reported in only one historical control study in the males (6/44 = 13.6%) and in none of the historical control females.

D. Discussion:

Comments:

This study was conducted between February 27, 1979 and February 20, 1981 in Japan and under Japanese sponsorship, had nothing to do with satisfying data requirements for the U.S. EPA, but was recently submitted to EPA by ICI Americas, Inc., Wilmington, DE. The original report, written in Japanese, has also been submitted along with an English translation.

Most of the submission is handwritten and, therefore, generally difficult to read. The report also contains omissions and ambiguities such as:

1. It is reported on page 12 that "there was only one death in the first half of the study (from the start of treatment to week 52)." Yet, tables on pages 33 and 34 show no deaths during that period.

2. It is stated on page 17 that testicular epithelioma was a frequent lesion and the incidence is quoted in the summary data on page 19. Yet, none was reported

[2] Biology and Diseases of Mice. R.O. Jacoby and J.G. Fox. In Laboratory Animal Medicine; J.G. Fox, B.J. Cohen, and F.M. Loew, Editors; Academic Press (1984); page 82.

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in Table 12A (page 65), Table 12C (page 66), Table 12E (page 69), Table 12G (page 74), Table 14 (page 85), and in the individual data on pages 88 through 101.

3. "Food ingested by individual mouse" is reported on pages 326 through 365. Yet, at the bottom of every page, data concerned with food consumption are summarized for rats.

4. Water intake by individual mice is reported on pages 367 through 406. Yet, each page is entitled "Water intake by individual rats" and "Experimental animal: rat."

5. Neoplastic lesions were reported together with non-neoplastic lesions and each lesion was reported only in terms of numerical incidence and not also in terms of percentage incidence.

6. None of the histopathological lesions was analyzed statistically.

7. No reference was made to MTD, whether or not it was reached. (Considering increased mortality and statistically significant changes in hematology, clinical chemistry, and organ weights at the 100 ppm level in both sexes, an MTD was reached.)

A very striking thing about this study is the apparent perfection with which it was performed. Not a single parameter designated for testing ever failed during the entire course of this study. Not a single blood sample was lost, weight determination missed, tissue not examined or autolyzed.

Considering that this study is not a major mouse oncogenic study (another study conducted by ICI has already been evaluated and accepted), the above missing data will not be required.

Systemic NOEL = 30 ppm (males and females)*
Systemic LEL = 100 ppm (males and females; HDT. See page 1 for findings).

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*Although absolute and relative weights of left adrenals were statistically significantly decreased in males from the 30 ppm group at the week 26 scheduled sacrifice, no weight changes were observed in right adrenals or in both adrenals at other scheduled sacrifices. The observed weight changes at week 26 do not, therefore, appear to be treatment-related.

Oncogenic NOEL = > 100 ppm (males and females).

Classification: Chronic feeding study: Core-Supplementary**

Oncogenic study: Core-Supplementary**

**Because of omissions and ambiguities listed above, a higher core classification cannot be assigned to this study.

15909:I:Locke:C.Disk:KENCO:2/8/88:DD:VO:LF:DD:VO:CB